

## FBS20- EZ1 Advanced XL – DNA Extraction

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### 1. Scope

- 1.1. This procedure describes the isolation of deoxyribonucleic acid (DNA) from biological specimens recovered from evidentiary and reference items for nuclear DNA typing using the EZ1 Advanced XL Robots.

### 2. Background

- 2.1. The EZ1 Advanced XL and the EZ1 DNA Investigator Kit purifies genomic DNA from 1 to 14 samples at a time, in lysis volumes of 200  $\mu$ L or 500  $\mu$ L, depending on the amount and condition of the starting material. DNA is isolated from lysates through its binding to the silica surface of the magnetic bead particles in the presence of a chaotropic salt. The magnetic bead particles are then separated from the lysates using a magnet. The DNA is then washed and eluted in TE (Tris EDTA) buffer or water.
- 2.2. The following protocols are validated for use:
  - 2.2.1. Trace – 200  $\mu$ L lysis volume, no substrate in tube
  - 2.2.2. Tip Dance – 200  $\mu$ L lysis volume, small cutting of substrate in tube
  - 2.2.3. Large Volume – 500  $\mu$ L lysis volume

### 3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

- 3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.
- 3.3. Do not use bleach on the inside of the EZ1 instrument or to directly clean-up spills from the Qiagen EZ1 DNA Investigator Kit. All spills will first be thoroughly washed with soap and water.

## 4. Materials Required

- 4.1. Qiagen EZ1 DNA Investigator Kit:
  - 4.1.1. Reagent cartridges (DNA Investigator)
  - 4.1.2. Disposable filter-tips
  - 4.1.3. Disposable tip-holders
  - 4.1.4. Sample tubes (2.0 mL)
  - 4.1.5. Elution tubes (1.5 mL)
  - 4.1.6. Buffer G2
  - 4.1.7. Proteinase K (20 mg/mL)
  - 4.1.8. Carrier RNA (cRNA) (310 µg)
- 4.2. Additional Reagents:
  - 4.2.1. Qiagen Buffer MTL (for 500 mL lysis volume protocol only)
  - 4.2.2. Digest Buffer (FBR35)
  - 4.2.3. Proteinase K (10 mg/mL) (FBR36)
  - 4.2.4. 1.0 M DTT (FBR38)
  - 4.2.5. TE Buffer
  - 4.2.6. Autoclaved Deionized Water (diH<sub>2</sub>O) (FBR06)

## 5. Standards and Controls

- 5.1. At least 1 reagent blank (RB) (i.e., extraction control) must be prepared and processed in parallel with each set of evidentiary specimens processed for DNA typing purposes. The RB(s) is comprised of all the reagents used in the analytical process and is carried through the same extraction, quantitation, amplification and detection procedure(s) as the evidence samples. For extraction sets exceeding 14 samples, the extraction procedure may be divided into sequential runs on the same robot, with at least 1 RB included in each run.
- 5.2. For differential extractions, the RB created in conjunction with the isolation of the epithelial cell fraction is designated as the non-sperm fraction (BK#.E in Sample

Tracking and Control Solutions (STACS)). The RB created in conjunction with the isolation of the male fraction is designated as the sperm fraction (BK#.S).

- 5.3. If a recombined sample (pellet and substrate) from p30 or sperm search testing is to be extracted, its associated Negative Control(s) will be processed in addition to the extraction RB. The Negative Control(s) will be created in STACS as an RB and include a unique identifier in the Lot Number location to differentiate it from the extraction RB. This Negative Control will be comprised of all the reagents used to prepare the sample for serology testing in addition to all the reagents used in the analytical process. It will be carried through the same lab processes, as applicable, as the evidence sample with which it is associated.
- 5.4. Samples suspected of containing low levels of DNA should be extracted separately from samples suspected of containing high levels of DNA whenever practicable.
- 5.5. To maintain a separation in time and space between questioned and known samples:
  - 5.5.1. At no time will questioned and known samples be simultaneously incubating in the same thermomixer or heat block.
  - 5.5.2. At no time will questioned and known samples be simultaneously extracted in the same instrument.
- 5.6. The RB will always be the last sample processed in a set. Any RBs created must match the volume of the most concentrated sample.
- 5.7. Any non-disposable utensil used to cut or manipulate the substrate must be cleaned with 10% bleach followed by 70% ethanol or sterile water after each use.
- 5.8. Any disposable utensil used to cut or manipulate the substrate will be discarded after each use.
- 5.9. All work surfaces, utensils, and instruments used must be cleaned as necessary with 10% bleach. A 70% ethanol or sterile water rinse must follow for all metal items. Alternatively, if using razor blades, a new blade will be used for each cutting.
- 5.10. The cRNA must be reconstituted prior to use. Add 310  $\mu\text{L}$  of TE Buffer to the lyophilized cRNA and vortex to mix to obtain a 1.0  $\mu\text{g}/\mu\text{L}$  solution.

**NOTE:** Store the reconstituted cRNA at  $-20^{\circ}\text{C}$ . Do not freeze-thaw the reconstituted cRNA more than 3 times.

- 5.11. Perform all appropriate instrument maintenance (e.g., daily and weekly) before beginning any EZ1 Advanced XL instrument run.
- 5.12. After completion of any EZ1 Advanced XL instrument run remove and clean the tray, tube and cartridge rack with 70% ethanol followed by diH<sub>2</sub>O. Replace them into the instrument.

## 6. Procedures

- 6.1. **Non-differential samples:** This procedure may be performed in either 200 µL or 500 µL lysis volumes. The larger lysis volume is needed for large substrates or to remove inhibitors. Non-differential 200 µL lysis protocol starts at 6.1.1. Non-differential 500 µL lysis protocol starts at 6.1.29.

### Non-Differential 200 µL Lysis:

- 6.1.1. Preheat thermomixer to 56°C.
- 6.1.2. Place each sample into a labeled, sterile tube.
- 6.1.3. If using the Trace protocol add samples to a non-QIAGEN 2.0 mL microcentrifuge tube.
- 6.1.4. If using the Tip Dance protocol, ensure that only a small cutting (e.g., ~3 mm x 3 mm cutting from a saturated blood card, ~1/4 cutting of a buccal swab) is added directly to the 2.0 mL skirted EZ1 tube.  
**NOTE:** Only use Tip Dance protocol for non-consumed reference samples.
- 6.1.5. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.
- 6.1.6. Add 190 µL of Buffer G2 and 10 µL Qiagen Proteinase K (20mg/mL) to each tube containing sample. Vortex well and quick-spin down.  
**NOTE:** The Buffer G2 and Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.
- 6.1.7. Incubate/shake on the thermomixer at 56°C and 900 rpm for a minimum of 2 hours to overnight. After digestion, vortex and quick-spin down the sample tubes.
- 6.1.8. **Trace Protocol Only:** Transfer the substrate to a filterless basket in a 2.0 mL microcentrifuge tube and spin at maximum speed for 2 minutes. Remove the spin basket, with substrate, and discard.
- 6.1.9. **Trace Protocol Only:** Transfer each lysate (approximately 200 µL) to a labeled 2.0 mL skirted EZ1 tube labeled with the tube number.

- 6.1.10. Add 1.0  $\mu\text{L}$  (1.0  $\mu\text{g}/\mu\text{L}$ ) reconstituted cRNA to each EZ1 tube. Vortex well and quick-spin down.
- 6.1.11. Turn on the robot (the switch is located on the back left hand side of the robot, just above the electrical cord) and then press “Start” to go to the main menu. “Investigator Protocols” will appear on the screen.
- 6.1.12. When using the EZ1 Advanced workstation three prompts will occur prior to reaching the main menu.
- 6.1.13. Select “Start” to begin run.
- 6.1.14. Select “ESC” to opt out of generating a QC report.
- 6.1.15. If performing the trace extraction, select the “Trace” protocol. If performing a tip dance extraction, select the “Trace TD” protocol.
- 6.1.16. Select “TE” as the elution solution.
- 6.1.17. Select “50  $\mu\text{L}$ ” as the elution volume.
- 6.1.18. The screen prompts that follow will summarize the selections and prompt a verification of positions of reagents, samples and plastics on the worktable.
- 6.1.19. Gently lift the front metal piece (tube rack) and place to the side. Gently lift the second metal piece (cartridge rack) located closer to the back of the robot and place to the side.
- 6.1.20. Insert the appropriate number of EZ1 Reagent Cartridges, corresponding to the number of samples to be extracted into the cartridge rack. After sliding each EZ1 cartridge all the way in, gently push down on the front plastic tab of the cartridge so that it makes a slight clicking sound. Once all EZ1 Reagent Cartridges are loaded, return the cartridge rack back to the original position in the robot.
- 6.1.21. Return the front metal piece (tube rack) into the robot. Remove the caps from the EZ1 tubes containing the sample lysates. Place each of these tubes into Row 4. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** Save the caps for disposal of tubes after the run is complete.
- 6.1.22. Place the plastic EZ1 tip holder into Row 2. The total number of tip holders will equal the number of samples to be extracted.
- 6.1.23. Place a filter tip into each of the tip holders from 6.1.22.
- 6.1.24. Label clean, sterile 1.5 mL elution tubes with, at a minimum, the sample name and tube number or associated bar code along the side of the tube and tube number on the cap of the tube. NOTE: Labeling may be conducted prior to this step. **The order and labeling of the elution tubes, as well as the loading of the tubes on the EZ1 (see previous and subsequent steps), must be witnessed by a second trained individual.**

- 6.1.25. Place the labeled 1.5 mL elution tubes into Row 1. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** The total number of elution tubes will equal the number of samples to be extracted. Retain the labeled sterile caps on a Kimwipe in the same orientation as the corresponding tube (these will be used once the extraction is completed by the robot). See image of work table in Appendix 13.2.

**NOTE:** Ensure that 1.5 mL and *not* 2.0 mL elution tubes are used.

- 6.1.26. Follow the prompts on the screen to close the instrument door and begin the run.

Refer to Appendix 13.1 for a list of messages displayed on the LCD during the robotic extraction.

- 6.1.27. After completion of the “Trace” or “Trace TD” protocol (~18 minutes), remove each tube from the EZ1 and add the correctly labeled screw cap to each tube.

**NOTE:** If elution volume is less than 50 µL obtain permission from TL to bring up the volume to 50 µL with TE from well 8 of the associated reagent cartridge.

- 6.1.28. Store the samples frozen.

### **Non-Differential 500 µL Lysis:**

- 6.1.29. Preheat thermomixer to 56°C.

- 6.1.30. Place each sample into a labeled, sterile 2.0 mL microcentrifuge tube.

- 6.1.31. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

- 6.1.32. Add 480 µL of Buffer G2 and 20 µL Qiagen Proteinase K (20mg/mL) to each tube. Vortex well and quick-spin down.

**NOTE:** The Buffer G2 and Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

- 6.1.33. Incubate/shake on the thermomixer at 56°C and 900 rpm for a minimum of 2 hours to overnight. After digestion, vortex and quick-spin down the sample tubes.

- 6.1.34. **Swabs or Cuttings Only:** Transfer the substrate to a filterless basket in a 2.0 mL microcentrifuge tube and spin at maximum speed for 2 min. Remove the spin basket, with substrate, and discard.

- 6.1.35. Add 400 µL Buffer MTL and 1.0 µL (1.0 µg/µL) reconstituted cRNA to each EZ1 tube. Vortex and quick-spin down.

**NOTE:** The Buffer MTL and reconstituted cRNA may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

- 6.1.36. Transfer each lysate (approximately 500 µL) to a 2.0 mL skirted EZ1 tube labeled with the tube number. Vortex briefly and quick spin down.
- 6.1.37. Turn on the robot (the switch is located on the back left hand side of the robot, just above the electrical cord) and then press “Start” to go to the main menu. “Investigator Protocols” will appear on the screen.
- 6.1.38. When using the EZ1 Advanced workstation three prompts will occur prior to reaching the main menu.
- 6.1.39. Select “Start” to begin run.
- 6.1.40. Select “ESC” to opt out of generating a QC report.
- 6.1.41. Select the “Large Volume” protocol.
- 6.1.42. Select “TE” as the elution solution.
- 6.1.43. Select “50 µl” as the elution volume.
- 6.1.44. The screen prompts that follow will summarize the selections and prompt a verification of positions of reagents, samples and plastics on the worktable.
- 6.1.45. Gently lift the front metal piece (tube rack) and place to the side. Gently lift the second metal piece (cartridge rack) located closer to the back of the robot and place to the side.
- 6.1.46. Insert the appropriate number of EZ1 Reagent Cartridges, corresponding to the number of samples to be extracted into the cartridge rack. After sliding each EZ1 cartridge all the way in, gently push down on the front plastic tab of the cartridge so that it makes a slight clicking sound. Once all EZ1 Reagent Cartridges are loaded, return the cartridge rack back to the original position in the robot.
- 6.1.47. Return the front metal piece (tube rack) into the robot. Remove the caps from the EZ1 tubes containing the sample lysates. Place each of these tubes into Row 4. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** Save the caps for disposal of tubes after run is completed.
- 6.1.48. Place the plastic EZ1 tip holder into Row 2. The total number of tip holders will equal the number of samples to be extracted.
- 6.1.49. Place a filter tip into each of the tip holders from 6.1.48.
- 6.1.50. Label clean, sterile 1.5 mL elution tubes with at minimum the sample name and tube number or associated bar code along the side of the tube and tube number on the cap of the tube. NOTE: Labeling may be conducted prior to this step. **The order and labeling of the elution tubes, as well as the loading of the tubes on the EZ1 (see previous**

**and subsequent steps), must be witnessed by a second trained individual.**

- 6.1.51. Place the pre-labeled 1.5 mL elution tubes into Row 1. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** The total number of elution tubes will equal the number of samples to be extracted. Retain the pre-labeled sterile caps on a Kimwipe in the same orientation as the corresponding tube (these will be used once the extraction is completed by the robot). See image of work table in Appendix 13.2.

**NOTE:** Ensure that 1.5 mL and *not* 2.0 mL elution tubes are used.

- 6.1.52. Follow the prompts on the screen to close the instrument door and begin the run.

Refer to Appendix 13.1 for a list of messages displayed on the LCD during the robotic extraction.

- 6.1.53. After completion of the “Large Volume” protocol (~18 minutes), remove each tube from the EZ1 and add the correctly labeled screw cap to each tube.

**NOTE:** If elution volume is less than 50  $\mu$ L obtain permission from TL to bring up the volume to 50  $\mu$ L with TE from well 8 of the associated reagent cartridge.

- 6.1.54. Store the samples frozen.

- 6.2. **Differential samples:** The “Large Volume” - 500  $\mu$ L lysis volume protocol is used.

### **Differential 500 $\mu$ L Lysis:**

- 6.2.1. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

- 6.2.2. To each sample tube, pipette 400  $\mu$ L Digest Buffer and 12  $\mu$ L of Proteinase K (10 mg/mL) solution. Vortex and quick-spin down in a microcentrifuge.

**NOTE:** The Digest Buffer and Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

- 6.2.3. Incubate the samples using a thermomixer or heat block either at 37°C for 2 hours to overnight or at 56°C for 1 hour.

- 6.2.4. After digestion, vortex and quick-spin down the sample tubes. Transfer the substrate to a filterless basket in a 2.0 mL microcentrifuge tube and spin for 3 – 5 minutes at maximum speed. Discard substrate and basket.



- 6.2.5. Remove the supernatant from the samples, being careful not to disturb the sperm pellet (approximately 50 µL remaining in the tube). Place the supernatant, or the non-sperm fraction (EF), into a new appropriately labeled microcentrifuge tube or 2.0 mL EZ1 skirted tube, if processed immediately on the EZ1.

**NOTE:** EF tubes can be processed immediately on the EZ1 (see step 6.2.14.) or can be stored at 4°C (for a maximum of overnight) for simultaneous processing with sperm fraction (SF).

- 6.2.6. Wash the sperm pellet by re-suspending in 500-1000 µL of TE Buffer or diH<sub>2</sub>O. Vortex and spin the samples in a microcentrifuge for 3-5 minutes at maximum velocity. Remove and discard the supernatant fluid, being careful not to disturb the cell pellet (up to 50 µL may be left in the tube).

- 6.2.7. Repeat Step 6.2.6 an additional 2 times for a total of 3 washes of the sperm pellet.

**NOTE:** The wash step can be repeated an additional 1 to 5 times depending upon the nature of the sample.

- 6.2.8. After the final spin, remove and discard all but approximately 50 µL of the supernatant.

- 6.2.9. **OPTIONAL:** Resuspend the pellet within the remaining 50 µL of supernatant by gently mixing the sample with a pipette. Remove approximately 4 µL of the sample and spot it on a glass microscope slide. Heat fix cells to the microscope slide following Step 6.7 of FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain.

- 6.2.10. **OPTIONAL:** Perform a Christmas Tree Stain using the technique described in Steps 6.8 through 6.15 of FBS07- Microscopic Examination of Spermatozoa by Christmas Tree Stain. Proceed to Step 6.2.11 if no epithelial cells are observed. However, if any intact epithelial cells remain, re-digest the sperm pellet by following these additional steps.

6.2.10.1. Add 400 µL of Digest Buffer to re-suspend the sperm pellet.

6.2.10.2. Add 12 µL of Proteinase K (10 mg/mL). Mix gently.

6.2.10.3. Incubate using a thermomixer or heat block at 37°C for 1 hour.

6.2.10.4. Spin at a maximum velocity for 3-5 minutes in a microcentrifuge. Remove and discard all but approximately 50 µL of the supernatant.

6.2.10.5. Re-suspend the pellet in 500 µL of Digest Buffer and vortex. Spin the sample 3-5 minutes in the microcentrifuge at maximum velocity. Remove and discard all but approximately 50 µL of the supernatant. Proceed to Step 6.2.11.

- 6.2.11. To the sperm pellet add 400 µL Buffer G2, 12 µL 1M DTT, and 15 µL Qiagen Proteinase K (20mg/mL).

**NOTE:** The Buffer G2 and Proteinase K may be added to the sample and incubated using the thermomixer or heat block at 37°C or 56°C while performing the Christmas Tree Stain. Once a slide is confirmed as containing no epithelial cells, the DTT may be added and the incubation continued.

**NOTE:** The Buffer G2, Qiagen Proteinase K and DTT may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the day.

6.2.12. Preheat thermomixer to 56°C.

6.2.13. Mix the samples gently and incubate using the thermomixer for 2 hours to overnight.

6.2.14. Add 400 µL Buffer MTL and 1.0 µL (1.0 µg/µL) reconstituted cRNA to both the EF and SF tubes. Vortex and quick-spin down.

**NOTE:** If EF tubes were stored at 4°C, allow lysates to equilibrate to room temperature prior to processing.

**NOTE:** The Buffer MTL and reconstituted cRNA may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

6.2.15. Transfer each lysate (approximately 900 µL) to a 2.0 mL skirted EZ1 tube labeled with the tube number. Vortex briefly and quick-spin down.

6.2.16. Turn on the robot (the switch is located on the back left hand side of the robot, just above the electrical cord) and then press “Start” to go to the main menu. “Investigator Protocols” will appear on the screen.

6.2.17. When using the EZ1 Advanced workstation three prompts will occur prior to reaching the main menu.

6.2.18. Select “Start” to begin run.

6.2.19. Select “ESC” to opt out of generating a QC report.

6.2.20. Select the “Large Volume” protocol.

6.2.21. Select “TE” as the elution solution.

6.2.22. Select “50 µl” as the elution volume.

6.2.23. The screen prompts that follow will summarize the selections and prompt a verification of positions of reagents, samples and plastics on the worktable.

6.2.24. Gently lift the front metal piece (tube rack) and place to the side. Gently lift the second metal piece (cartridge rack) located closer to the back of the robot and place to the side.

6.2.25. Insert the appropriate number of EZ1 Reagent Cartridges, corresponding to the number of samples to be extracted into the cartridge rack. After sliding each EZ1 cartridge all the way in, gently push down on the front plastic tab of the cartridge so that it makes a slight clicking sound. Once

all EZ1 Reagent Cartridges are loaded, return the cartridge rack back to the original position in the robot.

- 6.2.26. Return the front metal piece (tube rack) into the robot. Remove the caps from the EZ1 tubes containing the sample lysates. Place each of these tubes into Row 4. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** Save the caps for disposal of tubes after run is completed.
- 6.2.27. Place the plastic EZ1 tip holders into Row 2. The total number of tip holders will equal the number of samples to be extracted.
- 6.2.28. Place a filter tip into each of the tip holders from 6.2.27.
- 6.2.29. Label clean, sterile 1.5 mL elution tubes with at minimum the sample name and tube number or associated bar code along the side of the tube and tube number on the cap of the tube.

**NOTE:** Labeling may be conducted prior to this step. **The order and labeling of the elution tubes, as well as the loading of the tubes on the EZ1 (see previous and subsequent steps), must be witnessed by a second trained individual.**

- 6.2.30. Place the pre-labeled 1.5 mL elution tubes into Row 1. **Loading of the tubes on the EZ1 must be witnessed by a second trained individual.** The total number of elution tubes will equal the number of samples to be extracted. Retain the pre-labeled sterile caps on a Kimwipe in the same orientation as the corresponding tube (these will be used once the extraction is completed by the robot). See image of work table in Appendix 13.2.

**NOTE:** Ensure that 1.5 mL and *not* 2.0 mL elution tubes are used.

- 6.2.31. Follow the prompts on the screen to close the instrument door and begin the run.

Refer to Appendix 13.1 for a list of messages displayed on the LCD during the robotic extraction.

- 6.2.32. After completion of the “Large Volume” protocol (~18 minutes), remove each tube from the EZ1 and add the correctly labeled screw cap to each tube.

**NOTE:** If elution volume is less than 50 µL obtain permission from Technical Leader(s) (TLs) to bring up the volume to 50 µL with TE from well 8 of the associated reagent cartridge.

- 6.2.33. **OPTIONAL:** Spin tubes at maximum speed for 1 minute to pellet any remaining magnetic material and transfer the 50 µL extract to a new labeled 1.5 mL tube for storage, avoiding any pelleted material at the bottom.

**NOTE:** If optional step is not performed, spin tubes prior to amplification to avoid introduction of magnetic material into the amplification reaction.

6.2.34. Store the samples frozen.

## 7. Sampling

7.1. Not applicable

## 8. Calculations

8.1. Not applicable

## 9. Uncertainty of Measurement

9.1. Not applicable.

## 10. Limitations

- 10.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.
- 10.2. The separation of non-sperm and sperm cell DNA into their respective fractions is not always complete. It is not unusual for sperm cell DNA to be observed in the non-sperm (or epithelial) cell fraction and vice versa. The number of intact cells recovered in a sample and their capacity to endure the abrasive conditions of the differential extraction method is dependent upon the quality of the biological material being tested and the environmental conditions to which it has been subjected. The detection of residual DNA within a given fraction does not prohibit the use of the DNA typing results from that fraction.
- 10.3. The presence of nuclear DNA in the sperm fraction of a differential extraction is not always a dependable method of determining whether semen or spermatozoa in a particular biological specimen is present. Many factors may influence this determination such as differing profiles in the sperm fraction and non-sperm fraction of the sample and the associated semen screening results (AP, p30, microscopic sperm identification).

## 11. Documentation

11.1. Applicable STACS documentation

## 12. References

- 12.1. EZ1 DNA Investigator Handbook (Document Control Number: 4833)
- 12.2. Developmental Validation of QIAGEN EZ1® Robotic Platform And DNA Investigator® Silica-based Extraction Chemistry Version
- 12.3. Sterile Deionized Water (FBR06)
- 12.4. Digest Buffer (FBR35)
- 12.5. 10 mg/mL Proteinase K in 10mM Tris pH8.0 (FBR36)
- 12.6. 1.0 M Dithiothreitol (DTT) (FBR38)
- 12.7. Microscopic Examination of Spermatozoa by Christmas Tree Stain (FBS07)
- 12.8. FBU Quality Assurance Manual (FBQA01)

## 13. Appendix

13.1. The following steps are displayed and performed by the EZ1 Advanced XL during the DNA Investigator Kit runs (times are approximate):

13.1.1.	Protocol started	
13.1.2.	Piercing foil	17 of 18 min left
13.1.3.	Collecting elution buffer	
13.1.4.	Collecting beads	16 of 18 min left
13.1.5.	Resuspending beads	
13.1.6.	Collecting lysate	15 of 18 min left
13.1.7.	Binding	14 of 18 min left – 9 of 18 min left
13.1.8.	Binding: magnetic separation	9 of 18 min left
13.1.9.	Wash 1	8 of 18 min left
13.1.10.	Wash 1: magnetic separation	7 of 18 min left
13.1.11.	Wash 2	6 of 18 min left
13.1.12.	Wash 2: magnetic separation	
13.1.13.	Wash 3	5 of 18 min left
13.1.14.	Wash 3: magnetic separation	
13.1.15.	Rinse	4 of 18 min left
13.1.16.	Checking temperature	
13.1.17.	Elution	3 of 18 min left – 1 of 18 min left
13.1.18.	Protocol finished	

### 13.2. Image of Worktable (Image from DNA Investigator Handbook)



**Figure 4. Typical EZ1 worktable.**

1. First row: Elution tubes (1.5 ml) are loaded here.
2. Second row: Tip holders containing filter-tips are loaded here.
3. Third row: Tip holders containing filter-tips are loaded here. (In some protocols, this row is empty or loaded with 2 ml Sarstedt tubes.)
4. Fourth row: Sample tubes (2 ml) are loaded here.
5. Reagent cartridges are loaded into the cartridge rack.
6. Heating block with 2 ml tubes in the reagent cartridges for lysis.